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# Dynamics of *Streptococcus agalactiae* Colonization in Women during and after Pregnancy and in Their Infants

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**The population dynamics of *Streptococcus agalactiae* (group B streptococci [GBS]) colonization of the vagina and anorectal area was investigated in a cohort of 77 Danish women during and after their pregnancy by a new sensitive method. The mean carriage rate among individual observations was 36%, and the cumulative carriage rate over the entire observation period was 54%. Examination of more than 1,500 GBS isolates by pulsed-field gel electrophoresis demonstrated that the GBS population was remarkably homogeneous and stable in each carrier. Virtually all carriers were colonized by a single GBS clone on all occasions spanning up to 2 years. Repeated detection of the same clone even in women who were recorded as intermittent carriers suggests that the actual carrier rate exceeds 50% but that fluctuations in the GBS proportions of the flora occasionally preclude their detection. Newborns and young infants usually carried the same GBS clone as their mothers. However, only twice were identical clones of GBS detected in different women in contrast to the observed clonal relationships of clinical isolates. These observations strongly suggest differences in the properties and epidemiology of virulent GBS clones compared to clones commonly carried by healthy individuals.**

Infection by *Streptococcus agalactiae* (group B streptococci [GBS]) is still a common cause of neonatal diseases such as pneumonia, septicemia, and meningitis, although the incidence has declined in some countries as a result of active prevention efforts. The overall incidence of early-onset disease in 1998 to 2000 was 0.5 to 0.6 cases per 1,000 live births (12, 25, 30, 32), although there are geographical and racial differences (11). An incidence of 0.24 per 1,000 live births of proven GBS infections in neonates and young infants has been reported for Denmark in the past (8). It is generally accepted that bacterial colonization of the child during its passage through the birth canal is the main cause of early-onset infections among children aged less than 7 days. Intrapartum chemoprophylaxis offered to pregnant carriers is, therefore, the strategy now recommended by the Centers for Disease Control to reduce neonatal GBS infection (13). GBS also causes invasive infections in nonpregnant and pregnant adults (6, 35), although the incidences are lower than for newborns.

The carriage rates of GBS among pregnant and nonpregnant women and the rate of neonatal acquisition of GBS has been examined in the past (for reviews, see references 4, 13, and 33). However, relatively little is known about the kinetics of GBS colonization among individual carriers.

The main purpose of the present longitudinal study was to evaluate the prevalence of GBS colonization among pregnant and nonpregnant women by using a new sensitive differential agar medium (mixed blood [MB] agar) (19) and to examine the complexity and stability of the GBS population in individual women and their newborns. This was achieved by determination of the carriage and the clonal diversity of GBS defined by pulsed-field gel electrophoresis (PFGE) in a cohort of Danish women during pregnancy and 1 year after delivery.

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## MATERIALS AND METHODS

**Clinical samples.** The project was initiated January 1999 with approval from the Ethics Committee, County of Aarhus, Aarhus, Denmark, and after informed consent had been provided by all volunteers. The study was conducted from May 1999 to June 2001.

A cohort of 77 healthy women (aged 22 to 39 years; mean, 29 years) was monitored in a longitudinal study from the 16th week of gestation until shortly before delivery at the Department of Obstetrics, Aarhus University Hospital, Skejby, Denmark. All pregnancies were normal, and no complications due to GBS were observed. The participating women were instructed in the technique for taking bacteriological swabs at home. Carbon-containing cotton swabs on plastic sticks (Statens Serum Institut, Copenhagen, Denmark) were used for the sampling. Vaginal and anorectal swabs were obtained at different times during the pregnancy and for 48 of the participants once again 1 year after delivery (spring 2001). The data from four of these persons were omitted (see below). Sampling kits were shipped to each participant at regular intervals (ca. 3.5 weeks). The returned pairs of samples were grouped according to the gestation time of the sender. The exact number of subjects per group is given in figures and in tables.

Anorectal swabs and swabs from the ears, skin, and mouth were taken from newborns by one of the authors (S.M.H.) within 48 h after birth. On subsequent occasions, the mothers took fecal swabs from diapers of the 1-year-old infants.

Swab were immediately placed in Stuart's transport medium (Statens Serum Institut) and shipped to the laboratory by regular mail.

Culture-positive women without clinical risk factors do not receive intrapartum chemoprophylaxis in Denmark, and only four women from the study group were treated with antibiotics before delivery. The follow-up samples from these four participants were excluded from the analysis. One sample pair was excluded as one of the two swabs was missing. Two participants left the study after the first sample pair had been received.

**Cultivation of samples.** Swabs were carefully streaked on selective MB agar plates (see below) within 24 h after the samples were taken, and plates were incubated overnight at 37°C. Presumptive GBS colonies exhibiting characteristic partial hemolysis zones (19) were subcultured on 5% regular blood agar plates (Statens Serum Institut) and then stored at −70°C in 1 ml of nutrient beef broth plus 10% glycerol (Statens Serum Institut). If possible, 10 or more different GBS isolates from each swab were subcultured and stored. For control of the sampling technique, swabs were also streaked on regular nonselective blood agar plates.

The growth intensity of normal flora on the plates was evaluated as described below.

**Selective MB agar plates.** Plates containing antibiotics and mixed blood were prepared as described in detail elsewhere (19). Briefly, 1 liter of agar contained 40 g of blood agar base (CM854; Oxoid, Ltd., Basingstoke, United Kingdom), 2 g of sodium pyruvate, 0.247 g of $MgSO_4$, and 50 mg of L-cysteine hydrochloride monohydrate. The agar base was supplemented with 1 ml of a filter-sterilized (0.45-μm pore size) stock solution (8 mg/ml of $H_2O$) of gentamicin sulfate (G6896; Sigma-Aldrich, St. Louis, Mo.) and 1 ml of a filter-sterilized (0.45-μm pore size) stock solution (15 mg of 0.1 N NaOH/ml) of nalidixic acid (N8878; Sigma-Aldrich) per liter. For preparation of blood-containing top layers, 2.5% (vol/vol) sheep and 2.5% (vol/vol) horse red blood cells were added to the agar at 50°C. The blood cells were washed in phosphate-buffered saline and packed by centrifugation before use. Each plate (9 cm in diameter) was made of a lower layer of agar base without blood cells (25 ml) and a top layer of agar base (20 ml) containing the washed and mixed blood cells. The surface of each MB agar plate was treated with 200 μl of a sterile ready-made staphylococcus beta-toxin solution (19) before the plates were used.

**Bacterial identification.** Suspected GBS isolates were identified by a combination of standard tests (23). Strains were Gram stained, tested for catalase activity, and examined by the traditional CAMP test on CAMP plates (Statens Serum Institut). The presence of the group B antigen was examined by latex agglutination (Streptex ZL52; Murex Biotech, Ltd., Dartford, United Kingdom).

**Intensity of colonization evaluated on MB agar plates.** MB agar plates were used for semiquantitative evaluation of the degree of colonization as previously reported (19). Briefly, swabs were inoculated onto MB agar plates, followed by careful spreading by sets of streaks perpendicular to each other by using both sides of a sterile 10-μl plastic inoculation loop. The colonization intensity was evaluated semiquantitatively and blindly after incubation for 18 h at 37°C. Growth of GBS in streaking area 1 or 2 only was considered light colonization, whereas growth of GBS in one or more of the other streaking areas was considered moderate to heavy colonization. Samples without detectable growth of GBS were considered negative.

**Preparation of genomic DNA plugs.** Each GBS strain was inoculated in 2 ml of Todd-Hewitt broth (CM189; Oxoid) and incubated at 37°C for 14 to 16 h. The bacterial cells were collected by centrifugation. Supernatants were discharged, and each sediment was suspended in 0.5 ml of cold TE10:100 buffer (10 mM Tris-HCl, 100 mM EDTA [pH 8.0]). The suspensions were heated for 10 min at 50°C and then mixed with 0.5 ml of heat-melted 2% pulsed-field certified agarose (Bio-Rad Laboratories, Inc., Hercules, Calif.). The agarose suspensions were applied to disposable 10-well sample plug molds (Bio-Rad) and allowed to congeal. The agarose plugs were removed from the molds, transferred to 1 ml of lysis buffer (TE10:100 buffer, 1% Sarkosyl, $1.3 \times 10^4$ U of lysozymel [Roche, Basel, Switzerland]/ml, 100 U of mutanolysin [M9901; Sigma-Aldrich]/ml), and shaken gently for 2 h at 37°C. The lysis buffer was then replaced with a 1-mg/ml solution of proteinase K (catalog no. 705723 [Roche]) containing 0.5 M EDTA and 1% Sarkosyl (pH 8.0). The plugs were kept in the proteinase K solution at 50°C for 14 to 20 h. They were then washed in 2 ml of MilliQ water, and the proteinase was then inactivated by adding 1.5 ml of phenylmethylsulfonyl fluoride (PMSF) in TE10:1 buffer (10 mM Tris-HCl–1 mM EDTA [pH 8.0] plus 14 μl of PMSF stock solution [17 mg of PMSF/ml of isopropanol]/ml). The buffer was replaced with another 1.5 ml of PMSF-TE10:1 buffer after 30 min at room temperature. The plugs were kept in the new buffer for an additional 30 min. They were finally washed twice in 2 ml of MilliQ water and three times in 2 ml of TE10:1 buffer and then stored in the same buffer at 4°C.

**Cutting of DNA by restriction enzyme and PFGE.** The DNA-containing agar plugs (see above) were cut into three equal pieces. One piece from each plug was transferred to 1-ml Eppendorf tubes containing 10 U of *Sma*I restriction enzyme (656348; Roche) in 100 μl of 1× SurE/Cut buffer A (catalog no. 1417959; Roche). The DNA was digested for 16 h at room temperature. The processed agar plug pieces were then transferred to sample wells in an electrophoresis gel (30-well comb on a 21-by-14-cm gel, 1% pulsed-field certified agarose [Bio-Rad], and TBE buffer [0.045 M Tris-HCl, 0.045 M boric acid, 1 mM EDTA; pH 8.0]). The electrophoresis was performed in TBE buffer at 14°C on a GenePath strain typing system by using a CHEF-DRIII power module (Bio-Rad). The preprogrammed run condition "PSU" (initial ramp time, 5.3 s; final ramp time, 34.9 s; linear progression in ramp time; angle, 120° angle switching; voltage, 6 V/cm; running time, 19.5 h) was used for the pulsed field gel electrophoresis (PFGE).

The enzyme *Sma*I was unable to cut DNA from some GBS strains. Additional experiments with the restriction enzymes *Apa*I, *Not*I, *Sal*I, and *Xho*I were performed on these strains. The enzyme *Sal*I (catalog no. 348783; Roche) was found to give satisfactory results. *Sal*I was able to cut DNA from all *Sma*I resistant strains into a number of small fragments. Characteristic gel band patterns were seen for the strains when the preprogrammed run condition "DNA 25–250 kb" was used for the electrophoresis (initial ramp time, 1 s; final ramp time, 12 s; linear progression in ramp time; angle, 120° angle switching; voltage, 6 V/cm; running time, 18.5 h). Strains that were compared were tested on the same electrophoresis plate. After electrophoresis and ethidium bromide staining (0.5 μg per ml), the results were recorded by photography. The images were visually inspected, and strains exhibiting identical gel band patterns were considered belonging to the same clone.

**Carriage status.** Our previous study revealed a high degree of correlation between detection of GBS in anorectal and vaginal samples, but sampling from both sites increased the sensitivity of the method by ca. 10% (19). A sample pair was, therefore, considered negative if both samples in the pair (vaginal and anorectal swabs) were negative for growth of GBS and positive if one or both swabs were positive for growth of GBS.

The pregnant women were grouped according to the results from the examination of three or more sample pairs as persistent carriers, permanent noncarriers, or as intermittent carriers if GBS were detected in all, none, or some sample pairs, respectively. Persistent carriers were considered to be chronically colonized by GBS if the follow-up sample was found to be positive as well. Noncarriers were considered permanent noncarriers if all samples obtained during the observation period were negative for growth of GBS. The GBS colonization of intermittent carriers was considered transient if a positive sample pair was found on one occasion only.

**Reagents.** All chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) or from Sigma-Aldrich.

**Statistics.** STATA software (version 8; Stata Corp., College Station, Tex.) was used for the statistical analysis. The hypothesis of no association between gestational age and risk for carriage of GBS was tested by logistic regression with gestational age as a continuous variable. The hypothesis of no difference in the growth densities of GBS in anorectal and vaginal samples obtained from intermittent and persistent carriers was analyzed by ordered logistic regression. When the data involved more than one observation for each woman, robust standard errors were applied. Contingency tables were analyzed by using the Fisher exact test. Predictive values were calculated in relation to the time span between sampling points as the probability for the same test outcome for the sampling immediately before delivery (considered as "at delivery") compared to the outcome for a previous sampling. The positive predictive value (PPV) is calculated as the number of sample pairs found to be positive both at delivery and at the previous sampling divided by the total number of sample pairs found to be positive at the previous sampling. The negative predictive value (NPV) is calculated as the number of sample pairs found to be negative both at delivery and at the previous sampling divided by the total number of sample pairs found to be negative at the previous sampling.

## RESULTS

**Sampling procedure.** More than 3,000 isolates of GBS were recovered from 409 sample pairs (paired vaginal and anorectal swabs) obtained from a cohort of 77 women and from their infants over a 2-year period. The participants took the samples at home. The usefulness of this procedure was evaluated by streaking swabs on nonselective 5% blood agar plates. More that 95% of the rectal swabs and 75% of the vaginal swabs exhibited moderate to heavy growth of commensal bacteria (data not shown). The rest of the samples exhibited light growth densities. There was no correlation between the growth intensity of normal flora and the detection of GBS in the samples, and there was no differences among carrier groups (see below) in the distribution of samples exhibiting light or more heavy growth of normal flora. Thus, the sampling procedure seemed not to influence on the results.

**Carriage rates.** The initial sample pairs obtained during the 16 to 19 weeks of gestation were GBS positive for 38% of the participating women (Fig. 1). The GBS carriage rate varied from 33 to 38% (mean 36%) and was independent of the gestational age ($P = 0.83$). The rate was unchanged (34%) 1 year after delivery (Fig. 1 and 3).

The final samples from pregnant women were obtained dur-

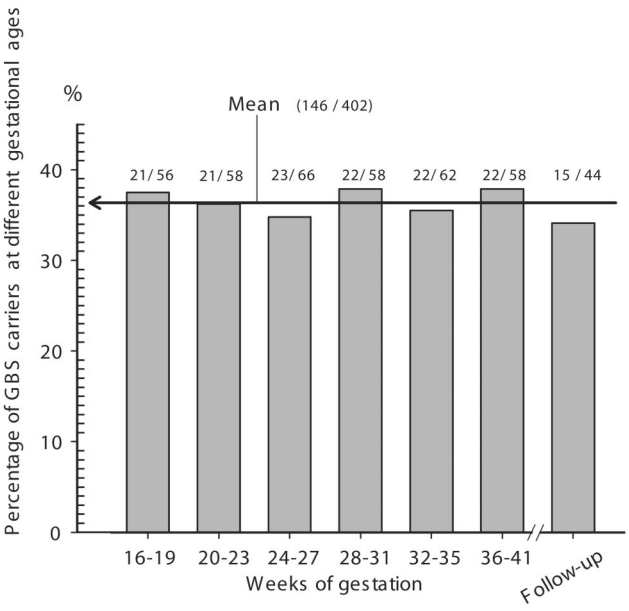


FIG. 1. Carriage rates of GBS in a cohort of 77 women during their pregnancy and 1 year after delivery. Figures indicate the number of women (carriers/total examined). There was no association between gestational age and carriage rate (mean, 36%; $P = 0.83$ [logistic regression]).

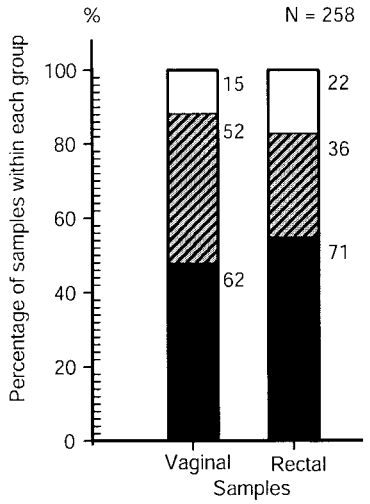


FIG. 2. Distribution of vaginal and rectal samples according to the density of GBS growth. Bars: □, negative samples; ▨, light growth of GBS; ■, moderate to heavy growth of GBS. Samples were obtained from 129 GBS positive sample pairs, i.e., one or both samples (vaginal and rectal) in the pair exhibited growth for GBS. GBS-negative samples were included since the corresponding vaginal or rectal samples were positive.

ing the eighth or ninth month of gestation. The time span between the initial and the final sampling point was ≥12 weeks for all participants (mean, 122 days). The repeated sampling (≥3 sample pairs per woman [mean, 4.6 pairs]; mean time span between samplings, 3.8 weeks) revealed that 53% of the women were permanent noncarriers since no sample pairs obtained during their pregnancy yielded growth of GBS (Table 1). Conversely, 28% of the pregnant women were found to be persistent carriers, since all sample pairs were positive for GBS. The remaining 19% of the pregnant women were classified as intermittent carriers, since only some of the sample pairs yielded growth of GBS. About half of the intermittent carriers were found to carry GBS at one occasion only; thus, the colonization of these carriers may be considered transient (Table 1). Some women changed colonization status over time. Therefore, the percentage of individuals allocated to the groups designated "noncarriers, intermittent carriers, or persistent carriers" is slightly different when the observation period was extended, as was the case for the follow-up group (see below).

TABLE 1. Carriage of GBS during pregnancy

| Group | No. of GBS-positive sample pairs obtained from each woman | No. of participants in each group with[a]: | | | |
|---|---|---|---|---|---|
| | | 3 sp/ woman | 4 sp/ woman | 5 sp/ woman | 6 sp/ woman |
| Permanent noncarriers | 0 | 2[a] | 13 | 20 | 5 |
| Transient colonization[b] | 1 | *0* | *2* | *1* | *3* |
| | 2 | *1* | *0* | *2* | *0* |
| | 3 | **3** | *1* | *0* | *0* |
| | 4 | | **5** | *1* | *2* |
| | 5 | | | **10** | *1* |
| | 6 | | | | **3** |

[a] The values indicate the number of participants in each group ($n = 75$). There were three or more sample pairs (sp; one vaginal and one rectal sample per pair; mean, 4.8 pairs) per woman. Regular typeface, permanent noncarriers ($n = 40$), 53%; italics, intermittent carriers ($n = 14$), 19%; boldface, persistent carriers ($n = 21$), 28%.

[b] See the text.

From the women grouped as intermittent carriers a total of 32 sample pairs positive for GBS were received. Of these 21 sample pairs (66%) yielded growth of GBS from both the anorectal and the vaginal swab. A higher proportion (84 of 97 [87%]) of positive sample pairs obtained from persistent carriers yielded GBS growth both from the vaginal and from the anorectal sample. The degree of GBS colonization was evaluated semiquantitatively for positive sample pairs (vaginal and/or rectal samples) obtained from persistent and intermittent carriers (Fig. 2). A considerable variation in the colonization intensity was observed for the individual women at different sampling points (data not shown), and no difference in the intensity of GBS colonization between persistent and intermittent carriers was observed for either vaginal ($P = 0.17$) or rectal ($P = 0.10$) samples.

At the follow-up examination performed approximately 1 year after delivery (mean, 16 months), sample pairs were obtained from 48 of the women, but 4 were excluded due to antibiotic treatment at delivery. Of the 44 women, 15 were found to be GBS positive (34%), whereas 29 were found to be noncarriers (66%). The percentage of carriers on this occasion was not different from that observed during pregnancy (Fig. 1 and 3). Of the 44 women from the follow-up group, 26 had been recorded as noncarriers during the pregnancy. Of these, 21 (81%) were still GBS negative at the follow-up, i.e., they were found to be permanent noncarriers. Thus, only five women (19%), who were noncarriers during pregnancy, had

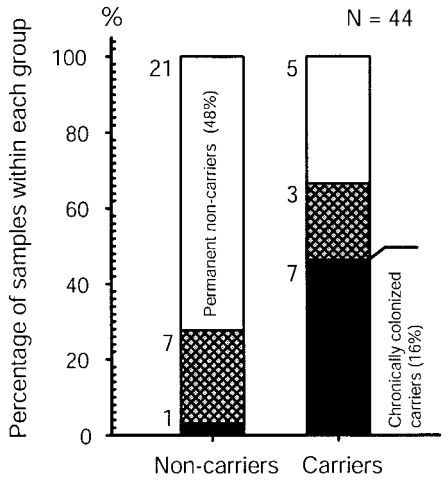


FIG. 3. Distribution of carriers and noncarriers of GBS one year after delivery compared to the carriage status during pregnancy. Follow-up samples from 44 women were examined. The carriage rate was 34% (15 of 44). Figures indicate the number of women per subgroup. Carriage status during pregnancy: □, noncarriers; ▨, intermittent carriers; ■, carriers. Persistent carriers, for whom all sample pairs were positive both during pregnancy and at follow-up, were considered chronically colonized (16%). Of the women in the follow-up group, 48% were found to be negative for GBS at all samplings occasions both during pregnancy and at follow-up, i.e., they were permanently noncolonized.

become detectably colonized by GBS during the subsequent 1 year period. Of eight women, who were persistent GBS carriers during pregnancy, only one had changed status to noncarrier during the intervening year, whereas seven (88%) were still carriers at the follow-up. These seven women were therefore considered chronically colonized since they were positive for GBS at all sampling occasions spanning more than a year and a half (mean, 636 days). Of 10 women who were found to be intermittent carriers during their pregnancy, 3 were GBS positive at the follow-up (Fig. 3).

Collectively, these results show that GBS were detected in about 50% of the examined pregnant and nonpregnant women from time to time and that 16% (7 of 44) of the women were chronically colonized at a detectable level for more than a year and a half (Fig. 3). Half of the intermittent carriers were positive for GBS at one occasion only and may be considered transient carriers. A total of 28% of the women were persistent carriers during their pregnancy (Table 1).

The predictive values of repeated samplings were calculated (see Materials and Methods). For a time-span of 3 to 8 weeks between sampling points, the PPVs and the NPVs were 0.84 and 0.89, respectively. For a time-span of 17 to 22 weeks, the corresponding values were 0.76 and 0.86, respectively. Thus, the GBS colonization status was stable for long periods of time in the majority of women.

Almost 50% (8 of 18) of newborns of mothers who were persistent carriers during pregnancy became detectably colonized with GBS during delivery. In comparison, only one newborn of a mother recorded as GBS negative and one child born by intermittently colonized mothers became colonized by GBS (Table 2). The same difference applies to 1-year-old infants (Table 3).

TABLE 2. Neonatal acquisition of GBS in relation to the mother's carriage status during pregnancy

| Status of the mother | No. of newborns ($n$ = 65) | |
|---|---|---|
| | Noncolonized (%) | Colonized (%) |
| Permanent noncarrier | 35 | 1 |
| Intermittent carrier | 10 | 1 |
| Persistent carrier | 10 | 8[a] |
| Total | 55 (85) | 10 (15) |

[a] Persistent carriers had a significant increased risk for transmitting GBS to the newborn compared to permanent noncarriers ($P < 0.001$ [Fisher exact test]). No differences were observed between the other groups.

**Colonization dynamics.** The colonization dynamic of GBS clones in the individual woman was elucidated by PFGE typing of GBS isolates recovered over time. By this analysis most isolates gave distinct gel band patterns, but DNA from some isolates was resistant to cutting by the *Sma*I enzyme. None of the restriction enzymes *Apa*I, *Xho*I, and *Not*I gave satisfactory results with these strains (not shown). However, the enzyme *Sal*I gave a range of small DNA fragments that were not separated well by PFGE when the PSU run conditions were used. Additional experiments with the same enzyme and different program parameters for the electrophoresis were performed. We found that the combination of *Sal*I digestion and the run condition "DNA 25–250 kb" for the electrophoresis yielded distinct gel band patterns for all of the *Sma*I-resistant strains. Thus, it was possible to recognize a DNA pattern for all GBS strains examined by the use of one of the two restriction enzymes. Strains exhibiting an identical gel band pattern after digestion with either of the two enzymes *Sma*I or *Sal*I were considered to belong to the same clone.

The clonal diversity of the GBS strains was investigated for each person. Up to 26 GBS isolates (mean of 11 vaginal plus 12 rectal strains per GBS-positive sample pair) obtained from the initial sample pair and from one or two of the following pairs were compared by PFGE. Six additional isolates (three vaginal and three rectal strains) from follow-up samples were also examined. A total of 1,564 isolates were analyzed by PFGE. Of the 35 intermittent or persistent GBS carriers (Table 1) 30 (86%) were colonized by only a single GBS clone at all occasions, i.e., the same clone was found in all positive samples from one person. Five women (14%) were carriers of two clones at the same time, one of these was subject 38 (see below). No clonal shift was observed in any of the women during 21 months observation period. Figure 4A shows an exceptional example. In this case the woman (subject 38) was colonized with two distinct GBS clones. One of these two

TABLE 3. Comparison of the carriage of GBS by 1-year-old infants and by their mothers

| Status of the mother | No. of infants ($n$ = 44) that were: | |
|---|---|---|
| | Noncarriers (%) | Carriers (%) |
| Noncarrier | 28 | 1 |
| Carrier | 11 | 4[a] |
| Total | 39 (89) | 5 (11) |

[a] Infants at age 1 year were more often carriers of GBS if their mothers were also carriers ($P = 0.039$ [Fisher exact test]).

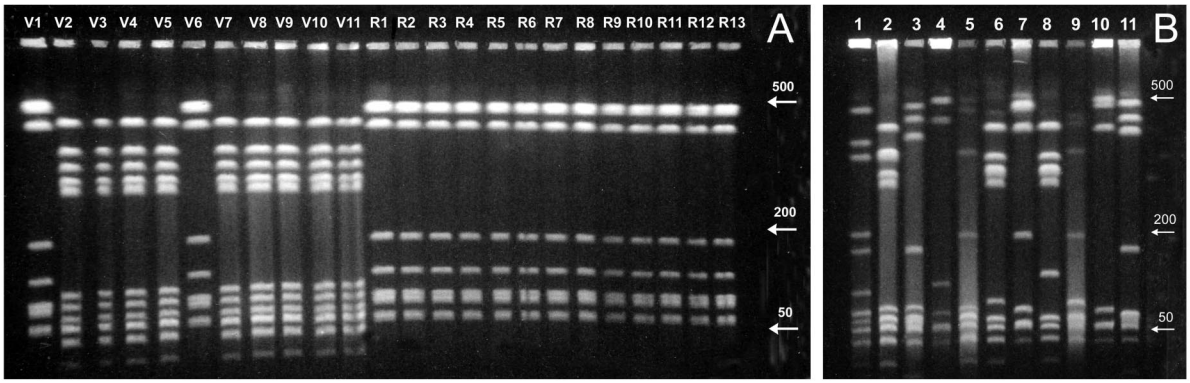


FIG. 4. PFGE gel band patterns for individual GBS isolates. The restriction enzyme *Sma*I was used for digestion of the DNA. (A) Twenty-four GBS isolates isolated from one sample pair obtained from one participant (subject 38). Only one clone was seen among the rectal isolates (R1 to R13). This clone was also seen among the vaginal isolates (V1 and V6), whereas the rest of the vaginal isolates (V2 to V5 and V7 to V11) belong to a different clone. (B) GBS isolates isolated from 11 different women. None of the 11 isolates exhibited an identical gel band pattern, i.e., they belonged to different clones.

clones was found in the vaginal sample only (Fig. 4A, V2 to V5 and V7 to V11), whereas the other clone was found both in the vaginal sample (Fig. 4A, V1 and V6) and in the rectal sample (Fig. 4A, R1 to R13). Both of the clones were detected in three additional sample pairs obtained from the same person over a 4-month period (results not shown). However, only one of these two clones was detectable in this women and in her child at the follow-up sampling 17 months later.

A total of 30 different gel band patterns were detected by PFGE among GBS isolates obtained from 32 women. Apparently identical GBS clones were found only two times in two different persons. Thus, most carriers carried a single "personal" clone of GBS (Fig. 4B). Exceptions included the newborns and the 1-year-old children, who in most cases (12 of 13) were found to be colonized by the same clone as their mothers. These results demonstrate that the clonal diversity was high in this cohort but was very limited for the individual person and that acquisition in infants was by vertical transmission from the mother.

## DISCUSSION

Besides being an important pathogen in neonates and in elderly individuals *S. agalactiae* is often part of the complex microflora of the human intestinal tract and vagina. However, the relationship between the healthy carrier state and disease is incompletely understood in terms of the significance of the colonization density, the diversity of virulence among clones of GBS, and the mechanisms of susceptibility to infection of some individuals. We used a recently developed sensitive differential agar medium that allows easy detection, semiquantitative evaluation, and representative recovery of GBS isolates (19) as a first step to obtain information relevant to elucidate these questions.

The application of the MB medium in the present study of GBS carriage in a cohort of 77 pregnant Danish women over a period of up to 2 years revealed detectable colonization in a mean of 36% (range, 33 to 38%; Fig. 1) at any observation point and a cumulative carriage rate of 54% (42 of 77) over the entire observation period. These figures are considerably higher than those (<15%) previously reported from Denmark and from several other countries (16, 20, 22, 24, 25, 36). Results comparable to ours have been reported from the United States (5, 14, 28). Although geographic, ethnic, and socioeconomic factors may influence the carriage rates, differences in sampling and culturing techniques probably contribute substantially to the variance of GBS carriage rates in different studies.

The fact that the application of a new differential medium to the study of GBS carriage in Danish pregnant women increased the observed carriage rate from <15 to 36% clearly underscores the significance of the sensitivity of the detection method and raises the question what the true carriage rate is. Studies of complex microbiotas such as those of the gut and oral cavity have clearly demonstrated the existence of multiple species that may escape detection even when semiselective culture techniques are used because of their low proportion of the flora. It has been suggested that there probably are more than 400 to 500 different organisms present at any time in the normal intestinal tract, although only about 100 different species have been isolated (17). It is also clear that proportions of individual species or clones of species may show temporary fluctuations and that such bacteria may become detectable as a result of various changes of the microenvironment (9, 24). The observation that some women (subjects 22, 32, and 70) who were found to be intermittently colonized carried the same clone on two or more occasions separated by a period without detectable GBS supports the assumption that the sensitivity of the detection method is a relevant parameter. Furthermore, by using MB agar for semiquantitative evaluation of growth densities it was demonstrated that 40% (52 vaginal and 36 rectal samples out of 221) of the positive samples exhibited only slight growth of GBS and that 29% (37 of 129) of the positive sample pairs exhibited growth from one of the two samples only (Fig. 2). Thus, it is conceivable that the true carriage rate for GBS is even higher than demonstrated in the present study and that GBS may indeed be a regular member of the microflora of the intestine and perianal area, as has been previously suggested by Easmon et al. (15).

We observed that women recorded as persistent GBS carri-

ers were more often detectably colonized at both the vagina and the rectum than were the intermittent carriers. However, samples from women recorded as persistent carriers did not yield more dense growth than samples from women recorded as intermittent carriers. Similarly, no relationship between prenatal carriage status and colonization intensity at delivery has been reported by others (7).

The results discussed above raise the question as to which factors may promote increases in the intestinal proportions of GBS and facilitate subsequent vaginal colonization to levels that may become clinically relevant. The results of the present study did not reveal any impact of the gestational age on the detectable GBS carrier status, and the carriage rate at the follow-up examination 1 year after delivery was not different from the carriage rate during pregnancy (Fig. 1). Thus, the proportion of women recorded as carriers was remarkably constant over time and the hormonal changes occurring at the initiation of pregnancy appear to have no impact on GBS carriage levels, although samples from the period before pregnancy were not available for confirmation of this assumption. In agreement with our observations, Anthony et al. (1) did not find any significant variation in the prevalence of GBS during pregnancy and in the intrapartum and postpartum periods. In contrast, Baker et al. found that the colonization rate almost doubles between the second trimester and delivery (2).

The colonization status was found to be relatively stable over long periods of time for most women (Table 1). Only 6 (18%) of 34 persistent carriers and noncarriers among the pregnant women had changed carrier status 1 year after delivery (Fig. 3). However, the women recorded as intermittent carriers (19%) reduced the accuracy of late antenatal cultures as a predictor for the GBS carriage status at delivery. Thus, a combination of vaginal and anorectal cultures obtained 3 to 8 weeks earlier was found to have a PPV for a current carriage status of 0.84, whereas the value declined to 0.76 if the time span was extended to 17 to 22 weeks. These figures are very similar to the values calculated by Yancey et al. (37).

Mothers who were GBS carriers often transmitted the bacteria to their children (Tables 1 and 2), as supported by the finding of clones that were indistinguishable (see below). This is in agreement with previous observations by others (3, 20, 27). Our material was too small to disclose whether density of colonization of the mothers influenced the vertical transmission to their children as observed by others (7). We found that 11% of the 1-year-old infants were carriers of GBS (Table 3). This relatively low carriage rate may, however, be due to sampling from feces in infant's diapers. Easmon et al. (15) reported that recovery rates of GBS were four times higher for rectal and perianal samples compared to fecal samples.

Genotyping of isolates by PFGE allowed us to examine the colonization dynamics of GBS in the studied cohort of women. The isolates were compared by PFGE by the use of the two restriction endonucleases *Sma*I and *Sal*I. These two enzymes were also selected by others as the best choice for PFGE characterization of GBS strains (3, 18, 31). The PFGE patterns showed that the GBS population was very uniform in all women examined. The majority of women carried a single GBS clone, and only a few were colonized by two different clones simultaneously (Fig. 4). The sample size and representativeness of isolates selected for further investigation are important factors for a correct estimation of the clonal diversity in a sample. We took these factors into account by performing multiple samplings from each subject over time and by PFGE typing of 5 to 25 GBS isolates from representative positive sample pairs. We cannot exclude that only the most dominant GBS clones are detected by this procedure and that other clones may "hide" below the detection limits. However, even if this is the case, the study demonstrates that one clone remains dominant over a period of more than 1 year. Helmig et al. (20) compared GBS isolated from positive samples obtained at sequential occasions by the use of multilocus enzyme electrophoresis and concluded that individual clones of GBS are carried vaginally for only limited periods of time. This conclusion was, however, based on relatively few observations. The results of the present study are in agreement with previous observations for intestinal populations of *Escherichia coli* and oral populations of *Streptococcus mitis* in adults, although those studies, in addition to stable clones, showed the existence of multiple clones that were only transiently detectable (9, 10, 21). The fact that stable GBS clones are not eliminated by the immune system suggest that they exists in a state of commensalism with the host.

Thus, the detectable GBS population was remarkably homogeneous and stable in the majority of the examined women. However, clones obtained from different individuals were mostly (28 of 32) distinct (Fig. 4B), a finding in agreement with results reported by Rolland et al. (31). Combined with the observation that approximately half of the women included in the present study never became detectable carriers, these findings indicate that acquisition of a new GBS clone after initial colonization at birth is a relatively rare event, although horizontal spread of GBS has been demonstrated between heterosexual college couples (26). Nevertheless, studies from the United States indicate that the major part of neonatal GBS type III infections in that country are caused by a single clone (29). Likewise, a single clone appears to predominate among the recently emerged cases of neonatal infections with serotype V both in North America and Europe (34). These observations suggest that certain clones are more readily disseminated in the population. The exact mechanisms accounting for apparent differences in the epidemiology of virulent clones and clones carried by healthy individuals are not clear. Identification of properties characteristic of particularly virulent clones may facilitate preventive measures both based on antenatal screening and immunization.

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